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08938468 PASCAL No.: 90-0106605
Detection and quantitation of interleukin-2 from individual cells
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In this report we present a technique for visualizing and quantitating IL-2 secreted from single cells. The procedure involves the attachment of cells to a protein-binding membrane such as Immobilon **PVDF** and the absorption by that membrane of secretory products. The membranes are treated with primary antiserum directed against the secreted product, with enzyme-conjugated secondary antiserum and substrate to produce a color reaction. Cells surrounded by zones of secreted product are visualized

Protein binding to nitrocellulose, nylon and **PVDF** membranes in immunoassays and electroblotting.

Tovey ER; Baldo BA

Kolling Institute, Royal North Shore Hospital, St. Leonards, Australia.

J Biochem Biophys Methods (NETHERLANDS) Aug-Sep 1989, 19 (2-3) p169-83

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A selection of different membranes commonly used to bind proteins in blotting and dot binding assays were investigated for a range of properties which would influence their performance. Large differences were observed in the membranes' ability to bind increasing amounts of protein, the effect of incubation times on the quantity of protein bound and the loss of proteins from the membranes following their incubation with different detergents or protein blocking agents. These differences could only partially explain the observed performance of the membranes when used as protein adsorbants in immunoassays and when different buffers were used for the electro-transfer of several different proteins to a range of membranes.

Descriptors: *Immunoassay--Methods--MT; *Immunoblotting--Methods--MT; *Membranes, Artificial; *Proteins--Analysis--AN; Antigens--Analysis--AN; Collodion; Indicators and Reagents; Kinetics; Nylons; Polyvinyls; Protein Binding; Radioimmunoassay--Methods--MT

CAS Registry No.: 0 (Antigens); 0 (Indicators and Reagents); 0 (Nylons); 0 (Poly

2392925

Detection and analysis of interferon- alpha receptors on plasma membranes and in detergent extracts.

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J. INTERFERON RES. vol. 10, no. 3, pp. 299-307 (1990.)

DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH

SUBFILE: Immunology Abstracts; Biochemistry Abstracts Part 1: Biological Membranes

We describe a simple, sensitive, and semiquantitative assay procedure to detect the presence of interferons- alpha (IFN- alpha) receptors on bovine spleen plasma membrane preparations or in detergent-solubilized extracts. The procedure involves spotting the sample on hydrophobic polyvinylidene difluoride (PVDF: Immobilon P) membranes, blocking the filter with milk, and binding radiolabeled IFN- alpha A to the membrane filter, with detection by either autoradiography or scintillation counting. This assay procedure has been applied for the identification of IFN- alpha receptors in crude and affinity-purified fractions. The partially purified IFN- alpha receptors have been further characterized by SDS-polyacrylamide gel electrophoresis (PAGE). The separated IFN- alpha receptor protein on the SDS-PAGE gel has been electrophoretically transferred to Immobilon membrane and visualized by ligand blotting. This provides an estimate of 95-110 kD for the apparent molecular weight and a tool for further studies of the receptor protein.

(Item 3 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
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9611056 BIOSIS Number: 94116056
RAPID SCREENING METHOD FOR POLYMORPHISM OF GROUP A APOLIPOPROTEINS
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J CLIN LAB ANAL 6 (5). 1992. 290-296. CODEN: JCANE
Full Journal Title: Journal of Clinical Laboratory Analysis
Language: ENGLISH

Polymorphism of apolipoproteins Al and All (apo Al and apo All) can be easily investigated in plasma by a simple method involving a 30-min incubation of EDTA plasma in the presence of urea, dithiothreitol, and Nonidet P-40 followed by subsequent isoelectric focusing (IEF). The sample (2 .mu.L) was applied to an ultrathin flat acrylamide gel of pH range 4-6, and focused using a Bio-Rad Mini IEF Cell for 1.5 h at a maximum of 500 V. Coomassie Blue R-250 was used to visualize the apolipoproteins. To verify the identity of the different apolipoproteins after IEF, the gel was immunofixed directly with anti-apo Al, or immunoblotted on polyvinylidene difluoride (PVDF) membrane using monospecific **antibodies** to apo Al and apo All and an anti-immunoglobulin-alkaline phosphatase conjugate. High-density lipoprotein (HDL) was used as a standard for Apo Al variants. Employing these techniques, human plasma apo Al was resolved into one major band (apo Al0, pl 5.54), and four minor bands identified as apo Al+2 (pl 5.75), apo Al+1 (pl 5.66), apo Al-1 (pl 5.45), and apo Al-2 (pl 5.34). Apo All was resolved into one major isoprotein designated as apo All0 (pl 4.87), and two minor isoforms apo All+1 and apo All-1 which focused at pls of 5.18 and 4.58, respectively. The results showed that these methods can be used to identify apo Al and All isoforms without prior ultracentrifugation to isolate the HDL. The entire procedure, including IEF, fixation (chemical or immunofixation), and staining, can be accomplished in 5 h compared to 2 days using previously reported technique. The identification and characterization of human apolipoprotein Al and All isoforms is important in clinical practice, e.g., diagnosis of tangier disease, and may be useful in studying structure-function relationships of these apoproteins.

Descriptors/Keywords: HUMAN HIGH DENSITY LIPOPROTEIN ANALYTICAL METHOD
Concept Codes:

- *10006 Clinical Biochemistry; General Methods and Applications
- *10056 Biochemical Methods-Lipids
- *10064 Biochemical Studies-Proteins, Peptides and Amino Acids
- *10066 Biochemical Studies-Lipids
- *13012 Metabolism-Proteins, Peptides and Amino Acids
- 10504 Biophysics-General Biophysical Techniques

Biosystematic Codes:

86215 Hominidae

Super Taxa:

Animals; Chordates; Vertebrates; Mammals; Primates; Humans